

WHAT IS CLAIMED IS:

1. A method of determining the suitability of at least one unfertilized oocyte for storage, said method comprising the steps of:

(a) providing an analysis of the first polar body from said unfertilized oocyte

5 which indicates the desirability of storing said oocyte; and

(b) storing or discarding said unfertilized oocyte based upon the results from said analysis of said polar body.

2. The method of claim 1, wherein said unfertilized oocyte is stored.

3. The method of claim 1, wherein said unfertilized oocyte is discarded.

10 4. The method of claim 2, further comprising (c) providing said stored, unfertilized oocyte for use in a fertility or reproductive treatment.

5. The method of claim 4, wherein the fertility treatment is in vitro fertilization.

6. A method of determining the suitability of at least one unfertilized oocyte for storage, said method comprising the steps of:

15 (a) providing the first polar body associated with an unfertilized oocyte;

(b) evaluating the first polar body to determine the desirability of storing said oocyte;

(c) storing or discarding said oocyte based upon the evaluation of said polar body.

7. A method of determining the suitability of at least one unfertilized oocyte for storage, 20 said method comprising the steps of:

(a) providing a first polar body associated with an unfertilized oocyte and a second first polar body associated with a second unfertilized oocyte;

(b) evaluating at least one first polar body associated with an unfertilized oocyte to determine the desirability of storing said oocyte; and

(c) storing or discarding said oocyte associated with said evaluated polar body based upon the evaluation of said evaluated polar body.

5 8. The method of claim 7, wherein (a) three or more first polar bodies are provided, each first polar body associated with a different unfertilized oocyte.

9. The method of claim 7 or 8, further comprising

evaluating all or some subset of said first polar bodies, each associated with one unfertilized oocyte, to determine the desirability of storing said oocytes associated with the 10 evaluated first polar bodies;

comparing said evaluations

storing all, some, or one of said oocytes based upon the comparison of the evaluations of said associated first polar bodies.

10 10. The method of claim 9, wherein at least one oocyte is discarded based upon the 15 comparison of the evaluations of said associated first polar bodies.

11. The method of claim 1, wherein at least one oocyte determined suitable for storing is stored in a manner appropriate for later use in fertility or reproductive treatment.

12. The method of claim 11 wherein storage of the oocyte comprises the following steps:

20 (a) microinjecting into the cytoplasm of said oocyte a protective agent which (i) comprises a sugar, (ii) is substantially non-permeating with respect to mammalian cell membranes, and (iii) maintains the viability of said cell such that it can be stored in a temporarily dormant state and restored to an active state; and

(b) treating said cell to cause it to enter the dormant state; and

(c) storing said cell in said dormant state.

13. The method of claim 1, wherein at least one oocyte determined unsuitable for storing is discarded.

14. A method of selecting at least one unfertilized oocyte for use in fertility or reproductive treatment, said method comprising the steps of:

5 (a) providing an analysis of the first polar body from said unfertilized oocyte which indicates the desirability of storing said oocyte; and

(b) storing or discarding said unfertilized oocyte based upon the results obtained from said evaluation of said polar body;

to thereby select an unfertilized oocyte for use in fertility or reproductive treatment.

10 15. The method of claim 14, wherein said unfertilized oocyte is stored.

16. The method of claim 15, wherein storing the unfertilized oocyte comprises the following steps:

15 (a) microinjecting into the cytoplasm of said unfertilized oocyte a protective agent which (i) comprises a sugar, (ii) is substantially non-permeating with respect to mammalian cell membranes, and (iii) maintains the viability of said cell such that it can be stored in a temporarily dormant state and restored to an active state; and

(b) treating said cell to cause it to enter the dormant state; and

(c) storing said cell in its dormant state.

20 17. The method of claim 16, further comprising contacting said cell with an extracellular protective agent that is substantially non-permeating with respect to mammalian cell membranes and that stabilizes the cell membrane of said cell.

18. The method of claim 16, wherein said protective agent comprises at least one sugar selected from the group consisting of sucrose, trehalose, fructose, dextran, and raffinose.

19. The method of claim 16, wherein said protective agent comprises at least one sugar selected from the group consisting of glucose, sorbitol, mannitol, lactose, maltose, and stachyose.

20. The method of claim 16, wherein said protective agent comprises at least one sugar with a glass transition temperature greater than -50.degree. C.

5 21. The method of claim 20, wherein said protective agent comprises at least one sugar with a glass transition temperature greater than -30.degree. C.

22. The method of claim 16, wherein said protective agent comprises at least one sugar with a molecular weight greater than 120 daltons.

10 23. The method of claim 16, wherein said protective agent comprises at least one sugar with a glass transition temperature greater than -30.degree. C. and a molecular weight greater than 120 daltons.

24. The method of claim 16, wherein said protective agent comprises a glycolipid or a glycoprotein that comprises at least one sugar moiety derived from a sugar with a glass transition temperature greater than -50.degree. C.

15 25. The method of claim 16, wherein the cytoplasmic concentration of said sugar is less than or equal to about 1.0 M following step (a) and prior to step (b).

26. The method of claim 25, wherein the cytoplasmic concentration of said sugar is less than or equal to about 0.2 M following step (a) and prior to step (b).

20 27. The method of claim 17, wherein said extracellular protective agent comprises an extracellular sugar.

28. The method of claim 27, wherein said cell is maintained in a liquid medium, and wherein the extracellular concentration of said extracellular sugar is less than or equal to about 1.0 M following dilution into said liquid medium.

25 29. The method of claim 28, wherein the extracellular concentration of said extracellular sugar is less than or equal to about 0.2 M following dilution into said liquid medium.

30. The method of claim 27, wherein said cell is maintained on a solid medium, and wherein the concentration of said extracellular sugar is less than or equal to about 1.0 M following administration to said cell.

31. The method of claim 30, wherein the concentration of said extracellular sugar is less
5 than or equal to about 0.2 M following administration to said cell.

32. The method of claim 16, wherein step (b) comprises freezing said cell to a cryogenic temperature.

33. The method of claim 32, wherein said cell is plunge frozen.

34. The method of claim 32, wherein said cell is cooled at a rate between 0.3 and
10 6.degree. C. per minute to a final temperature that is at least -50.degree. C.

35. The method of claim 34, wherein said cell is at a rate between 0.3 and 3.degree. C. per minute to a final temperature that is between -50 and -10.degree. C.

36. The method of claim 32, wherein further comprising step (d) wherein step (d) comprises thawing said cell.

15 37. The method of claim 16, wherein step (b) comprises drying said cell to a level sufficient to permit dry storage.

38. The method of claim 37, wherein step (b) comprises freeze drying said cell.

39. The method of claim 37, wherein step (b) comprises vacuum or convective drying said cell.

20 40. The method of claim 37, wherein step (d) comprises rehydrating said cell.

41. The method of claim 16, wherein only said protective agent is employed.

42. The method of claim 16, wherein prior to step (a), said cell is maintained in a hypertonic medium having an osmolarity greater than 300 mosm.

43. The method of claim 36, wherein following step (d), said cell is cultured in a hypertonic medium having an osmolarity greater than 300 mosm.

44. The method of claim 16, wherein a penetrating cryoprotectant mixture is added to said preservation agent.

5 45. A method of culturing a cell in vitro, comprising incubating said cell in a hypertonic medium having an osmolarity greater than 300 mosm.

46. The method of claim 45, wherein the osmolarity of said medium is greater than 320 mosm.

47. The method of either claim 14, wherein said unfertilized oocyte is discarded.

10 48. The method of either claim 14, further comprising removing said oocyte from storage and using said oocyte in a fertility or reproductive treatment.

49. A method of selecting at least one unfertilized oocyte suitable for storage, said method comprising the steps of:

15 (a) providing the results of an evaluation of a first polar body derived from said unfertilized oocyte;

· (b) storing or discarding said unfertilized oocyte on the basis of said results from the evaluation of said polar body;

to thereby select an oocyte suitable for storage.

50. The method of either claim 49, further comprising fertilizing a stored oocyte.

20 51. The method of claim 50, further comprising storing said fertilized oocyte or embryo.

52. The method of claim 51, wherein the freezing protocol comprises one or more of the following protocols:

53. The method of the above claims wherein the oocyte is a human oocyte.

54. The method of the above claims wherein the oocyte is non-human oocyte.

55. The method of any one of the above claims wherein the oocyte is taken from a prize animal.

56. The method of any one of the above claims where all steps are performed by a single party.

57. The method of any one of claims 1-5 wherein step (a) is performed by a first party, and step (b) is performed by a second party.

58. The method of claim 4, wherein steps (a) and (b) are performed by a first party and step (c) is performed by a second party.

10 59. The method of either claim 4, 6, or 7 wherein step (a) is performed by a first party, step (b) is performed by second party, and step (c) is performed by a third party.

60. The method of claim 5, 6, or 7 wherein steps (a) and (b) are performed by a first party and step (c) is performed by a second party.

15 61. The method of claim 5, 6, or 7, wherein step (a) is performed by a first party and a second party performs steps (b) and (c).

62. The method of either claim 5, 6, or 7, wherein step (a) is performed by a first party, step (b) is performed by a second party, and step (c) is performed by a third party.

63. The method of either claim 5, 6, or 7, wherein steps (a) and (c) are performed by a first party, step (b) is performed by a second party.

20 64. A method of selecting an unfertilized oocyte for use in a fertility or reproductive treatment, said method comprising the steps of:

(a) providing at least one unfertilized oocyte and a polar body from said oocyte;

(b) placing said oocyte and polar body in storage; and

(c) analyzing said polar body to determine the desirability of using said oocyte in a fertility or reproductive treatment.

65. A method of selecting an unfertilized oocyte suitable for use in fertility or reproductive treatment, said method comprising the steps of:

- 5 (a) providing an unfertilized oocyte and a polar body from said oocyte;
- (b) placing said oocyte and polar body in storage; and
- (c) analyzing said polar body at a later time to determine the desirability of using said oocyte in a fertility or reproductive treatment.

66. A method of scoring the suitability of an unfertilized oocyte for use in fertilization or 10 reproductive treatment, said method comprising the steps of:

- (a) providing at least one unfertilized oocyte and a polar body from said oocyte;
- (b) storing said oocyte; and
- (c) analyzing said polar body to determine the suitability of using said stored oocyte in reproductive treatment.

15 67. The method of claim 66, further comprising: (d) assigning a value, code, or location to the frozen oocyte which indicates the suitability of said oocyte for use in fertilization or reproductive treatment.

68. The method of either claim 66 or 67, wherein the treatment comprises in vitro fertilization.

20 69. A method of scoring the suitability of an unfertilized oocyte for use in reproductive treatment, said method comprising the steps of:

- (a) providing an unfertilized oocyte and a polar body from said oocyte;
- (b) storing said unfertilized oocyte and polar body; and

(c) analyzing said polar body to determine the suitability of using said oocyte in fertility/ reproductive treatment;

70. The method of claim 69, further comprising: (d) assigning a value, code, or location to the stored oocyte which indicates the suitability of using said oocyte in reproductive treatment.

5 71. The method of any one of claims 64, 65, 66, 67, or 69, further comprising entering into a database the results of the evaluation or an indicator of the suitability of said oocyte for use in a fertility or reproductive treatment and a means for correlating said results with said oocyte.

10 72. The method of either claim 68 or 70, further comprising entering into a database the code, value, or location indicating the suitability of said oocyte for use in a fertility or reproductive treatment and providing a means for correlating said code, value or location with said oocyte.

73. A method of retrieving from storage an oocyte suitable for use in a fertility or reproductive treatment, comprising

(a) accessing a database produced according to either claim 71 or 72; and

15 (b) retrieving said oocyte from storage if the results of said polar body evaluation in the database or alternatively if the code, value, or location assigned to said oocyte in the database indicates that the oocyte is suitable for use in said fertility or reproductive treatment.

74. The method of claim 73, wherein step (a) is performed by a first party and step (b) is performed by a second party.

20 75. The method of any one the above claims, wherein an evaluation or an analysis comprises determining if the polar body has one or more chromosomes above or below the normal chromosome number, to thereby evaluate the possibility that the associated oocyte has one or more chromosomes above or below the normal chromosome number.

25 76. The method of claim 75, wherein a determination that the polar body has less than the normal number of chromosomes causes the associated oocyte to be discarded.

77. The method of claim 75, wherein a determination that the polar body has more than the normal number of chromosomes causes the associated oocyte to be discarded.

78. The method of claim 75, wherein the determination that the polar body has the normal number of chromosomes causes a party to store the associated oocyte.

5 79. The method of claim 78, wherein the stored oocyte is used in a fertility or reproductive treatment.

80. The method of claim 75, wherein the test comprises

(a) providing an array of a plurality of probes capable of hybridizing with targeted chromosomal material;

10 (b) exposing said probes to the chromosomes from the first polar body under conditions that allow the hybridization of said probes to said target chromosomes;

(c) visualizing said probes

to thereby visualize any chromosomal abnormalities, such as the presence of duplicate chromosomes or the absence of chromosomes.

15 81. The method of claim 80, wherein the array of probes comprises at least one probe for at least one of the following chromosomes: 13, 18, 16, 21, 22 or the X chromosome.

82. The method of claim 75, wherein the evaluation or analysis consists of:

(a) providing at least one nucleotide primer capable of annealing to complementary chromosomal material;

20 (b) mixing said primer with the chromosomal material from a first polar body under conditions that would allow annealing of the primer to its complementary chromosomal material;

(c) adding an elongating mixture comprising a nucleotide polymerase and at least one labeled nucleotide in a mixture comprising at least four nucleotides, under conditions that would allow elongation of a hybridized primer.

5 (d) placing the chromosomal material under conditions that would allow visualization elongated primers hybridized to said chromosomal material.

83. The method of the above claims, wherein an evaluation or an analysis comprises determining if the polar body has one or more structural chromosomal abnormalities.

84. The method of claim 83, wherein the evaluation or analysis comprises:

10 (a) providing at least one nucleotide primer capable of annealing to complementary chromosomal material;

(b) mixing said primer with the chromosomal material from a first polar body under conditions that would allow annealing of the primer to its complementary chromosomal material;

15 (c) adding an elongating mixture comprising a nucleotide polymerase and at least one labeled nucleotide in a mixture comprising at least four nucleotides, under conditions that would allow elongation of a hybridized primer.

(d) placing the chromosomal material under conditions that would allow visualization elongated primers hybridized to said chromosomal material.

85. The method the above claims, wherein an evaluation or analysis of the first polar body comprises determining the presence or absence of one or more genetic disorder, e.g. one or 20 more single-gene disorder.

86. The method of claim 85, wherein the determination comprises amplifying a gene of interest or a region of interest in one or more genes from the first polar body.

25 87. The method of claim 85 or 86, wherein the gene or region of interest is from one of the following: a gene encoding CFTR, a gene encoding dystrophin, a Beta Thalassemia gene, a

gene encoding Factor VIII, a gene encoding Factor IX, Tay-Sachs gene, a survival motor neuron (SMN) gene, and a HD gene.

88. The method of claim 85 or 86, wherein the first polar body is evaluated for a genotype associated with one or more of the following genetic disorders:

5 Adenoleukodystrophy, Amyotrophic Lateral Sclerosis (ALS), Becker Muscular Dystrophy, Beta Thalassemia, Cerebellar Ataxia, Charcot-Marie-Tooth Disease, Chondrodysplasia
Aganglionic Megacolon, Conradi-Hunerman Syndrome, Cystic Fibrosis, Duchenne Muscular Dystrophy, Hemophilia A or B, Huntington's Disease, Fragile X Syndrome, Glycogen Storage Disease, Hirschsprung Disease, Ichthyosis, Lesch Nyhan, Myopathies, Polycystic Ovary
10 Syndrome, Restenosis Pigmentosa, Sickle cell Anemia, Tay-Sachs Disease, and Von Willebran Disease.

89. The methods of claims 85-88, wherein said evaluation of a polar body comprises a subtractive genotypic analysis of one or more genes or regions of a gene comprising:

15 (a) providing a determination of the presence or absence of one or more regions of a gene from said polar body of said unfertilized oocyte; and

(b) comparing the gene or region of the gene from the polar body to both of the genes or regions of the genes from diploid maternal genetic material as an indication of what genes or regions of genes are present in the oocyte;

wherein the oocyte contains the alternative gene of interest or region of interest to that
20 present in the polar body.

90. The method of claim 89, wherein the determination of the presence or absence of a gene or region of a gene comprises amplifying genetic material by PCR.

91. The method of claim 89, wherein the evaluation comprises providing genetic material from the polar body under conditions that would allow a probe specific to a particular 25 form of a gene to hybridize with that gene.

92. The method of claim 89, wherein the evaluation comprises a subtractive gene expression analysis.

93. The method of any one of the above claims, wherein an evaluation or analysis of the first polar body comprises determining the presence or absence of a particular allele or form of 5 at least one gene.

94. The method of claim 93, wherein said determination comprises amplifying at least one gene of interest or at least one region of interest in a gene from the polar body.

95. The method of claim 93, wherein said evaluation or analysis comprises a subtractive genotypic analysis of one or more genes or regions of a gene comprising:

10 (a) providing a determination of the presence or absence of one or more genes or regions of a gene from said polar body of said unfertilized oocyte; and

(b) comparing said gene or said region of a gene from the polar body to both of the genes or regions of the genes from diploid maternal genetic material as an indication of what genes or regions of genes are present in the oocyte;

15 wherein the oocyte contains the alternative gene or region of a gene to that present in the polar body.

96. The method of claim 95, wherein the determination of the presence or absence of a gene or region of a gene comprises amplifying genetic material by PCR.

97. The method of any one of claims 93-96, further comprising storing or discarding said 20 oocyte based on the results of at least one gene or region of a gene present in the polar body.

98. The method of any of the above claims, wherein an evaluation of a polar body comprises a subtractive gene expression analysis of polar body relative to the genotype of the oocyte.

99. The method according to any one of the above claims, wherein an evaluation of a 25 polar body consists of an analysis of first polar body morphology, said analysis comprising a

gradation of the first polar body according to one or more of the following criteria: shape, surface smoothness, fragmentation, and size of the perivitalline space.

100. A method of providing information regarding the suitability of an unfertilized oocyte for fertility or reproductive treatment, comprising:

5 (a) providing a polar body from an unfertilized oocyte; and
(b) amplifying all or part of the genome from said polar body

101. The method of claim 100, further comprising (c) evaluating the amplification product.

102. The method of claim 100, further comprising (c) storing the amplification product
10 for later evaluation, e.g. analysis after 1, 2, 3, 4, 5, 6 mos....1, 2, 3, 4, 5, 6, 10 years.

103. A method of providing information regarding the suitability of an unfertilized oocyte for use in fertility or reproductive treatment, comprising

(a) providing a polar body from an unfertilized oocyte;
(b) storing said polar body; and

15 (c) amplifying all or part of the genome from said stored polar body.

104. The method of claim 103, further comprising evaluating the amplified genome.

105. The method of claim 103, further comprising storing the amplified genome for later evaluation.

106. The method of any of the above claims, wherein an evaluation comprises
20 determining if said first polar body has one or more chromosomes above or below the normal chromosome number, to thereby evaluate the possibility that the associated oocyte has one or more chromosomes above or below the normal chromosome number.

107. The method of claim 106, wherein a determination that the polar body has less than the normal number of chromosomes causes the associated oocyte to be discarded.

108. The method of claim 106, wherein a determination that the polar body has more than the normal number of chromosomes causes the associated oocyte to be discarded.

5 109. The method of claim 106, wherein the determination that the polar body has the normal number of chromosomes causes a party to store the associated oocyte.

110. The method of claim 109, wherein the stored oocyte is used in a fertility or reproductive treatment.

111. The method of claim 106, wherein the test comprises

10 (a) providing an array of a plurality of probes capable of hybridizing with targeted chromosomal material;

(b) exposing said probes to the chromosomes from the first polar body under conditions that allow the hybridization of said probes to said target chromosomes;

(c) visualizing said probes

15 to thereby visualize any chromosomal abnormalities, such as the presence of duplicate chromosomes or the absence of chromosomes.

112. The method of claim 111, wherein the array of probes comprises at least one probe for at least one of the following chromosomes: 13, 18, 16, 21, 22 or the X chromosome.

113. The method of claim 106, wherein the evaluation or analysis consists of:

20 (a) providing at least one nucleotide primer capable of annealing to complementary chromosomal material;

(b) mixing said primer with the chromosomal material from a first polar body under conditions that would allow annealing of the primer to its complementary chromosomal material;

(c) adding an elongating mixture comprising a nucleotide polymerase and at least one labeled nucleotide in a mixture comprising at least four nucleotides, under conditions that would allow elongation of a hybridized primer.

5 (d) placing the chromosomal material under conditions that would allow visualization elongated primers hybridized to said chromosomal material.

114. The method of any one of the above claims, wherein an evaluation or analysis of the first polar body comprises determining the presence or absence of one or more genetic disorder, e.g. one or more single-gene disorder.

10 115. The method of claim 114, wherein the determination comprises amplifying a gene of interest or a region of interest in one or more genes from the first polar body.

116. The method of claim 114 or 115, wherein the gene or region of interest is from one of the following: a gene encoding CFTR, a gene encoding dystrophin, a Beta Thalassemia gene, a gene encoding Factor VIII, a gene encoding Factor IX, Tay-Sachs gene, a survival motor neuron (SMN) gene, and a HD gene.

15 117. The method of claim 114 or 115, wherein the first polar body is evaluated for a genotype associated with one or more of the following genetic disorders:

Adenoleukodystrophy, Amyotrophic Lateral Sclerosis (ALS), Becker Muscular Dystrophy, Beta Thalassemia, Cerebellar Ataxia, Charcot-Marie-Tooth Disease, Chondrodysplasia, Aganglionic Megacolon, Conradi-Hunerman Syndrome, Cystic Fibrosis, Duchenne Muscular Dystrophy, Hemophilia A or B, Huntington's Disease, Fragile X Syndrome, Glycogen Storage Disease, Hirschsprung Disease, Ichthyosis, Lesch Nyhan, Myopathies, Polycystic Ovary Syndrome, Restenosis Pigmentosa, Sickle cell Anemia, Tay-Sachs Disease, and Von Willebrand Disease.

20 118. The methods of claims 114-117, wherein said evaluation of a polar body comprises a subtractive genotypic analysis of one or more genes or regions of a gene comprising:

(a) providing a determination of the presence or absence of one or more regions of a gene from said polar body of said unfertilized oocyte; and

(b) comparing the gene or region of the gene from the polar body to both of the genes or regions of the genes from diploid maternal genetic material as an indication of what genes or regions of genes are present in the oocyte;

5 wherein the oocyte contains the alternative gene of interest or region of interest to that present in the polar body.

119. The method of claim 118, wherein the determination of the presence or absence of a gene or region of a gene comprises amplifying genetic material by PCR.

120. The method of claim 118, wherein the evaluation comprises providing genetic material from the polar body under conditions that would allow a probe specific to a particular 10 form of a gene to hybridize with that gene.

121. The method of claim 118, wherein the evaluation comprises a subtractive gene expression analysis.

122. The method of any one of the above claims, wherein an evaluation or analysis of the first polar body comprises determining the presence or absence of a particular allele or form 15 of at least one gene.

123. The method of claim 122, wherein said determination comprises amplifying at least one gene of interest or at least one region of interest in a gene from the polar body.

124. The method of claim 122, wherein said evaluation or analysis comprises a subtractive genotypic analysis of one or more genes or regions of a gene comprising:

20 (a) providing a determination of the presence or absence of one or more genes or regions of a gene from said polar body of said unfertilized oocyte; and

(b) comparing said gene or said region of a gene from the polar body to both of the genes or regions of the genes from diploid maternal genetic material as an indication of what genes or regions of genes are present in the oocyte;

wherein the oocyte contains the alternative gene or region of a gene to that present in the polar body.

125. The method of claim 124, wherein the determination of the presence or absence of a gene or region of a gene comprises amplifying genetic material by PCR.

5 126. The method of any one of claims 122-125, further comprising storing or discarding said oocyte based on the results of at least one gene or region of a gene present in the polar body.